REMARKS

On page 2 of the Office Action, the Examiner acknowledges Applicants' election of the invention of Group I, i.e., Claims 1-48, with traverse. However, the Examiner maintains that restriction is proper.

Applicants reserve the right to file a Petition under Rule 144 with respect to the Restriction Requirement.

In paragraph 3, on page 3 of the Office Action, the Examiner contends that Applicants' priority document does not support differential methylation in liver cancer tissue DNA extracts, i.e., Figure 9 of the present application is not found in the priority documents. Hence, the Examiner contends that the claims directed to liver cancer are not entitled to benefit of the April 23, 1999, filing date of the priority document.

Applicants submit that the priority document does disclose the method of the invention in relation to liver cancer (e.g., see page 6, lines 23-25; page 10, line 5; and page 21, lines 20-21 thereof).

In paragraph 5, on page 4 of the Office Action, the Examiner rejects Claims 1-16, 22-23 and 35-46 under 35 U.S.C. § 112, first paragraph.

Specifically, the Examiner states that while the specification is enabling for methods for detecting prostate or liver cancer by differential methylation analysis of the GST-Pi gene, such does not provide enablement for detecting <u>any</u> disease or condition based upon differential analysis of the GST-Pi gene.

The Examiner is requested to note that Applicants have limited the rejected claims such that they are directed to cancers characterized by abnormal methylation in the GST-Pi gene.

Accordingly, Applicants respectfully submit that the claims are enabled by the present specification, and thus request withdrawal of the Examiner's rejection.

In paragraph 6, on page 8 of the Office Action, the Examiner rejects Claims 1-48 under 35 U.S.C. § 112, second paragraph for reasons (A)-(E).

Regarding reason (A), the Examiner contends that the claims do not recite a final process step which associates the process with a method for diagnosing or prognosing the disease or condition.

In order to overcome this rejection, the Examiner suggests amending Claim 1 to recite ", wherein the detection of the amplified DNA is indicative of methylation, wherein the detection of methylation is indicative of the cancer".

Applicants have amended the Claim 1 essentially per the Examiner's suggestion.

Regarding reason (B), the Examiner states that it is unclear what is meant by "within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) GpG sites -43 to +55". The Examiner notes that this rejection can be overcome by amending Claim 1 to recite the nucleotide positions within a sequence ID number shown in the application.

The Examiner is requested to note that -43 to +55 refers to nucleotide sequences in Figure 1, which are presented in SEQ ID NOs:52-54. Thus, Applicants have amended Claims 1 and 35 to refer to SEQ ID NOs:52-54.

Regarding reason (C), the Examiner contends that the claims are indefinite in the recitation of "the amplifying step (ii)" because it is not clearly an amplifying step, but rather a step of exposing DNA to reactants and conditions for the purposes of amplification.

In view of the amendment to step (ii) of Claim 1 to recite, "carrying out amplification of said isolated DNA so as to amplify a target region...", Applicants respectfully submit that the Examiner's rejection has been met.

Regarding reason (D), the Examiner contends that Claims 4-7 and 23 are indefinite in the recitation "the treated DNA", because such lacks proper antecedent basis. The Examiner notes that Claim 2 refers to "treated DNA", and appears to suggest that Claim 4 should be dependent upon Claim 2, not Claim 3.

In view of the amendment to Claim 4 to place such dependent on Claim 2, Applicants respectfully submit that the Examiner's rejection has been met.

Regarding reason (E), the Examiner states that Claims 14 and 44 are indefinite in recitation of "including serum and lasma".

In view of the amendments to Claims 14 and 44, Applicants respectfully submit that the Examiner's rejection has been met.

Accordingly, Applicants respectfully submit that the claims clearly and definitely recite the invention of interest, and thus request withdrawal of the Examiner's rejection.

In paragraph 7, on page 11 of the Office Action, the Examiner rejects Claims 1, 3, 14-25, 35-39 and 44-47 under 35 U.S.C. § 102(e) as being anticipated by Lee et al.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

Contrary to the Examiner's contention, Lee et al does not disclose feature (ii) of present Claim 1. Claim 1 characterizes the amplification step as "being selective in that it only amplifies the target region if the said sites or sites at which abnormal cytosine methylation occurs is/are methylated". The amplification described in Lee et al is not selective, rather it relies on a preceding exhaustive digestion step, which itself is selective. If there were no prior digestion step, the amplification step of Lee et al would amplify the target region, irrespective of the methylation status of the cytosines, that is, the amplifying step of Lee et al *per se* is not selective.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested by Lee at al, and thus request withdrawal of the Examiner's rejection.

In paragraph 9, on page 12 of the Office Action, the Examiner rejects Claims 2, 4-13 and 40-41 under 35 U.S.C. § 103 as being unpatentable over Lee et al in view of Herman et al.

Specifically, the Examiner states that while Lee et al does not specifically teach the benefit of using bisulfite treatment for distinguishing methylated DNA from unmethylated DNA,

Herman et al teaches methylation specific PCR (MSP) for rapid identification of DNA methylation patterns in CpG containing nucleic acid (see the Abstract thereof). Hence, the Examiner concludes that it would have been obvious to modify the method of detecting methylation in the promoter region of Lee et al with the MSP methylation detection method of Herman et al to achieve the present invention.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

As discussed above, Lee et al does not teach or suggest the present invention. Further, for the following reasons, it is clear that Herman et al does not provide the deficiencies that exist therein.

Herman et al is completely silent in relation to the GST-Pi gene. Additionally, the method described in Herman et al is based on the use of amplification primers that are specifically designed to recognise CpG sites "to take advantage of the differences in methylation to amplify specific products to be identified by the invention assay" (see column 4, lines 56-59).

The primer of Herman et al is a CpG specific oligonucleotide (see column 5, lines 45-46) that "typically contains 12-20 or more nucleotides" and "is designed to be 'substantially' complimentary to each strand of the genomic locus to be amplified and includes the appropriate G or C nucleotides (see column 6, lines 38-41), These primers distinguish between methylated and non-methylated nucleic acid (see Claim 1, column 35, lines 61-66).

The Examiner finds significance in Herman et al's comment that the detection of the methylated CpG-containing nucleic acid

in the specimen may be indicative of cellular proliferative disorder or neoplasia including prostate cancer (see column 10, lines 48-57).

However, Herman et al's detection method is based on the use of primers that can distinguish between methylated and unmethylated CpG site(s), not abnormal methylation as claimed in the present application. It is inherent in the MSP method described in Herman et al that the CpG sites that can be recognized by the oligonucleotide primers are closely located to one another, simply because of the physical limitation of the length of the primer.

The Examiner contends that Lee et al teaches, *inter alia*, that using the PCR assay strategy resulted in more than 90% of human prostatic carcinoma DNA specimens analyzed exhibiting deoxycytidine methylation changes extensively encompassing the GST-Pi promoter region.

It is important to note that Lee et al's method is concerned with detecting extensive methylation in a region that encompasses the GST-Pi promoter region. In fact, Lee et al clearly indicates that the PCR technique described therein is used to amplify a fragment containing 12 recognition sites for HpaII/MspI (see the Abstract, Figure 1, first column, page 445). It can be seen from Figure 1 that if the sample DNA is cut at only one of 12 restriction sites in the sample DNA, the two-step PCR would not produce a PCR product. It is therefore clear that the detection method of Lee et al relies on detecting all 12 recognition sequences for HpaII and MSP1 in a sample DNA. This is confirmed in the sentence bridging pages 448 and 449 of Lee et al, which

indicates that modified GST-Pi promoter target sequences with fewer numbers of HpaII recognition sites (i.e., less that 12) could be included to monitor false positive results.

As can be seen from Figure 1, the first step PCR amplifies a region of approximately 605 base pairs. The second PCR step amplifies a 164 base pair region of the product of the first amplification step. Also, as can be seen from Figure 1, the 12 HpaII/MspI restriction are spread over several hundred base pairs.

If it were accepted, for argument's sake, that the ordinary artisan would have been motivated to modify the method of detecting methylation in the promoter region of Lee et al with the MSP method of Herman et al, what would be the result?

Herman et al's assay relies on the use of oligonucleotide primers that are designed to anneal to a methylated CpG site or sites that is/are a marker for disease states. As already mentioned, the methylated CpG sites detected by the oligomeric primers of Herman et al limits detected methylated CpG sites to those being in close proximity to each other.

Lee et al teaches a sequence having 12 recognition sites for HpaII/MspI as a marker for prostate cancer. These 12 recognition sites are spread over at least 500 base pairs. Therefore, applying the teachings of Herman et al to Lee et al, the MSP method would involve the use of a "primer" of several hundred nucleotides in length! It is highly unlikely that such a "primer" would be capable of distinguishing between methylated and unmethylated CpG sites at the 12 restriction sites taught by Lee et al.

In truth, the ordinary artisan would not have been motivated by Herman et al to use the MSP method in the assay of Lee et al. Herman et al teaches that the MSP technique can be used in cases where the methylated CpG site(s) is a marker for disease can be selectively annealed with an oligonucleotide primer. Lee et al's assay on the other hand is concerned with determining a marker that is extensive methylation (12 restriction sites) spread over many hundreds of base pairs. Therefore, the artisan would not have seen the relevance of Herman et al's MSP method based on oligonucleotide primers to the detection of the extensive methylation marker taught by Lee et al.

The present invention is based on Applicants' discovery that despite the teachings in Lee et al of a 12 restriction site fragment marker for prostate cancer, abnormal methylation at one or more CpG sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 can be used as a marker for cancer. It was only after this discovery that Applicants came to the realization that primers or probes that are selective for the at least one of the abnormally methylated sites could be used in an assay without the need for exhaustive digestion with restriction enzymes, as taught by Lee et al. Neither Lee et al nor Herman et al disclose or suggest that the CpG site(s) identified in the present application may be used as a marker for cancer.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested by Lee at al even when combined with the teachings of Herman et al, and thus request withdrawal of the Examiner's rejection.

In paragraph 10, on page 16 of the Office Action, the Examiner rejects Claims 26-29 under 35 U.S.C. § 103 as being unpatentable over Lee et al in view of Herman et al and in further view of Jhaveri et al and Morrow et al.

Specifically, the Examiner states that while neither Lee et al nor Herman et al teach the specific primers claimed for amplification of the CpG island of GST-Pi, the Examiner contends that Jhaveri et al teaches regions of GST-Pi which are methylated and Morrow et al teaches the full-length GST-Pi sequence which includes the proximol promoter and the first and second exon and intron. Hence, the Examiner concludes that it would have been obvious to have modified the primers taught by Lee et al to obtain additional primers for the amplification of the CpG island to achieve the primers of Claims 26-29.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

As discussed above, Lee et al when combined with Herman et al does not teach or suggest the present invention. Further, it is clear that Jhaveri et al and Morrow et al do not provide the deficiencies that exist therein.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested by Lee at al even when combined with the teachings of Herman et al, Jhaveri et al and Morrow et al, and thus request withdrawal of the Examiner's rejection.

In paragraph 11, on page 18 of the Office Action, the Examiner rejects Claims 30-34 and 48 under 35 U.S.C. § 103 as being unpatentable over Lee et al in view of Tchou et al.

Specifically, the Examiner states that while Lee et al does not specifically teach detection of hypermethylation in GST-Pi as an indication of liver cancer, Tchou et al teaches the role of GST-Pi expression in hepatocarcinogenesis. Hence, the Examiner concludes that it would have been obvious to modify the teachings of Lee et al for detecting prostate cancer based upon differential methylation of GST-Pi with the teachings of Tchou et al that GST-Pi is also hypermethylated in hepatocellular carcinoma to achieve the present invention.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

As discussed above, Lee et al does not teach or suggest the present invention. Further, it is clear that Tchou et al does not provide the deficiencies that exist therein.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested by Lee at al even when combined with the teachings of Tchou et al, and thus request withdrawal of the Examiner's rejection.

In view of the amendments to the claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

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A P P E N D I X

Marked-up Version of Changes

IN THE CLAIMS:

The claims are amended as follows:

Claim 1. (Amended) A diagnostic or prognostic assay for a [disease or condition] cancer in a subject, said [disease or condition] cancer characterized by abnormal methylation of cytosine at [site or sites within] at least one CpG site in a target region within the glutathione S-transferase (GST) Pi gene and/or it regulatory flanking sequences, wherein said assay comprises the steps of[;]:

(i) isolating DNA from said subject,

(ii) carrying out amplification of said isolated DNA so as to amplify [exposing said isolated DNA to reactants and conditions for the amplification of] a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the [disease or condition] cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) [determining] detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 represented by nucleotides 1-98 of SEQ ID NOS:52-54.

Claim 4. (Amended) An assay according to [Claim 3] Claim 2, wherein said amplification step comprises PCR amplification [utilises] utilizing a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 5. (Amended) An assay according to claim 4, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the [disease or condition] cancer being assayed.

Claim 7. (Amended) An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the [disease or condition] cancer being assayed.

Claim 11. (Amended) An assay according to claim 10, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA

of a subject with the [disease or condition] cancer being assayed.

Claim 13. (Amended) An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with [the disease or condition] cancer being assayed.

Claim 14. (Twice Amended) An assay according to Claim 1, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma [(including serum and plasma)], semen, urine, lymph, or bone marrow.

Claim 16. (Amended) An assay according to claim 1 [15], wherein the [disease or condition] cancer to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

Claim 17. (Amended) An assay according to claim 16, wherein the [disease or condition] cancer to be assayed is prostate cancer.

Claim 25. (Amended) An assay according to claim 17, wherein the [amplifying] amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

Claim 26. (Amended) An assay according to claim 17, wherein the [amplifying] amplification involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9)

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 27. (Amended) An assay according to claim 17, wherein the [amplifying] amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

Claim 28. (Amended) An assay according to claim 17, wherein the [amplifying] amplification step involves PCR amplification

using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

Claim 29. (Amended) An assay according to claim 17, wherein the [amplifying] amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 30. (Amended) An assay according to claim 16, wherein the [disease or condition] cancer to be assayed is liver cancer.

Claim 31. (Amended) An assay according to claim 30, wherein the [amplifying] amplification step is used to amplify a target

region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

Claim 34. (Amended) An assay according to claim 30, wherein the [amplifying] amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

Claim 35. (Amended) A diagnostic or prognostic assay for a [disease or condition] cancer in a subject, said [disease or condition] cancer characterised by abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of:[;]

(i) isolating DNA from said subject, and

(ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55 represented by nucleotides 1-98 of SEQ ID NOs:52-54.

Claim 44. (Twice Amended) An assay according to Claim 35, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma [(including serum and plasma)], semen, urine, lymph, or bone marrow.

Claim 46. (Amended) An assay according to claim 35 [45], wherein the [disease or condition] cancer to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

Claim 47. (Amended) An assay according to claim 46, wherein the [disease or condition] cancer to be assayed is prostate cancer.

Claim 48. (Amended) An assay according to claim 46, wherein the [disease or condition] cancer to be assayed is liver cancer.

Claims 15 and 45 are being cancelled.

New Claims 51-76 are being added.